NOTE TO FILE

BNF 031

Date: March 15, 1996

Subject: Male Sterile Corn

Keywords:

Corn; *Zea mays*; *Streptomyces hygroscopicus*; glufosinate ammonium tolerant; herbicide tolerant; *bar*; phosphinothricin acetyltransferase (PAT); *Bacillus amyloliquefaciens*; barnase (ribonuclease); male sterility, ampicillin resistance; *bla*.

Background

In a letter dated January 12, 1996, Plant Genetic Systems (PGS) submitted a summary of their safety assessment of a new hybrid seed production system (SeedLink™) in yellow dent corn. This production system is based on the presence of transformation event MS3.

Intended Effect and Food/Feed Use

PGS states that corn lines containing transformation event MS3 are male sterile which allows more efficient production of hybrid seed.

Corn is one of the world's primary cereal grain crops. Corn grain (kernels) has both animal feed and human food applications. Human food usage of the grain includes the production of high fructose corn syrup, glucose, corn oil, corn grits, and corn meal. Animal feed uses for the grain include the kernel itself which may be fed whole or in a processed form and byproducts from the production of human food, such as hominy feed or corn steep liquor. The entire plant may also be fed as green chop or in a fermented state as silage.

According to PGS, the modified corn line contains the *barnase* gene that encodes the enzyme barnase, a ribonuclease derived from *Bacillus amyloliquefaciens*. The *barnase* gene is selectively expressed in the tapetal cell layer of the anther, a cell layer that plays a vital nutritive role during pollen formation. Expression of the barnase enzyme in the tapetal cell layer disrupts production of RNA, and thus, the expression of protein in these cells. This disruption in protein expression in essence destroys the tapetal cell layer, rendering the anthers incapable of producing viable pollen grains. This inability to produce viable pollen grains renders the plant male sterile and provides reliable pollination control.

The *barnase* gene is linked to a glufosinate tolerance gene *(bar)* which encodes the enzyme phosphinothricin acetyltransferase (PAT). Pat was derived from *Streptomyces hygroscopicus* ATTC21705 and confers resistance to glufosinate ammonium, the active ingredient in several broad spectrum, non-selective herbicides. PAT is expressed in virtually all cells in the new corn variety. According to PGS, the linkage of the field selection system (i.e., PAT) to the pollination control system (i.e., barnase) enables the selection of male sterile lines independent of plant stage and provides quality assurance in hybrid seed production by allowing for efficient rogueing of fertile plants in a segregating population.

Molecular Alterations and Characterization

In the January 12, 1996, submission, PGS described the identity and function of the genetic material introduced into the modified corn. PGS reported that this line was developed by electroporation of enzymatically-treated immature embryos of inbred line H99 with plasmid pVE108, which contained two chimeric gene constructs: 1) the PTA29-*barnase*-3'nos gene construct, conferring male sterility and 2) the P35S-*bar*-3'nos construct, conferring herbicide tolerance. The PTA29 promoter region of the anther-specific gene TA29 from tobacco *(Nicotiana tabacum)* was used to limit the expression of barnase activity to anther cells whereas the 35S promoter region of the cauliflower mosaic virus was used to direct the expression of the PAT enzyme in all plant cells. Plasmid pVE108 also contains the *bla* gene under the control of a bacterial promoter. The *bla* gene confers ampicillin resistance by expression of a β-lactamase. Plasmid pVE108 was constructed and propagated in *Escherichia coli* WK6 in the presence of the helper plasmid pMc5barstar, which directed the expression of *barstar, a* specific inhibitor of barnase, which was needed to counter possible adverse effects of expressed barnase in the *E. coli* host. The plasmid pMc5barstar also conferred resistance to β-lactam and chloramphenicol antibiotics in the *E. coli* host. At the final growth step, chloramphenicol was omitted from the culture medium to reduce the amount of helper plasmid in the pVE108 DNA preparation, that was used in the genetic alteration of corn line H99.

PGS reported that there are two adjacent insertions of novel genetic material in the MS3 genome. The first is composed of two copies of plasmid pVE108 in a head-to-tail arrangement. The second site has one copy of pVE108 and a rearranged partial piece of the helper plasmid pMc5barstar, which included a nearly intact *barstar* sequence and a partial, rearranged copy of the *cat* gene. The two insertions are oriented in a tail-to-tail configuration with respect to the *bar* genes. PGS indicates that none of the genes except *bar* and *barnase* are expressed in MS3 corn as determined by the absence of mRNA coding

for the gene products. In addition, no Bla activity was detected in grain using a β-lactamase activity assay (detection limit-750 µg/kg).

PGS also provided data and information that allowed the firm to conclude that: 1) the insertions occurred at a single locus as determined by Southern blot analysis; 2) the insert is stably incorporated and is transmitted as a Mendelian dominant trait; and 3) the linkage between herbicide tolerance and male sterility is absolute (i.e., all herbicide tolerant plants are male sterile).

Safety of the Introduced Proteins

The introduced genetic material encodes two new proteins: barnase and PAT. PGS provided data and information that allowed the firm to determine that: 1) *bar* mRNA was present at detectable levels as determined by Northern blot analysis, in leaves and immature kernels, but was undetectable in roots, dry seeds, and germinating seeds; 2) PAT activity was undetectable in transformed grain using a PAT specific assay (detection limit-5 mg/kg); and 3) expression of the barnase enzyme is limited to the tapetal cell layer. PGS concluded that barnase can not be expressed outside the tapetal layer, since significant barnase activity destroys cells and the modified plants develop normally except for male sterility. The firm also was unable to detect barnase mRNA in grain. PGS stated that the processing of corn to yield corn oil, high fructose corn syrup, and alcohol, the major corn-derived products for human consumption, would be expected to completely degrade any residual protein, if present, in these products. PGS concluded that growing and consuming corn grain derived from the transformed corn line would not result in significant human or animal exposure to the PAT or barnase enzymes.

PGS nevertheless discussed the safety for human and animal consumption of these proteins. PGS stated that the PAT enzyme: 1) is sensitive to proteases and therefore would be rapidly degraded in the gastrointestinal tract; 2) is completely inactivated at a pH less than 5.0 and therefore would be inactivated in the acidic stomach of humans and animals; and 3) showed no significant sequence homology to known allergens present in several sequence databases. PGS also stated that barnase showed no significant sequence homology to known allergens and that ribonucleases, such as barnase, are naturally expressed in all plant tissues and therefore are already part of human and animal diets.

PGS also stated that it had observed no indications that any metabolic change had occurred in the transformed plants which would lead to new plant products that could be consumed, particularly by animals fed the entire plant.

Nutritional Assessment

GRAIN

PGS presented data to support their conclusion that there were no significant differences in the composition of grain samples derived from male sterile plants and those from male fertile corn. Analytes included amounts of moisture, starch, total protein, and crude fat. The firm also reported that there were no significant differences in the distribution of amino acids or fatty acids between MS3 and its non-modified counterpart.

SILAGE

PGS also presented data to support their conclusion that there were no significant differences in the composition of silages made from a MS3 hybrid, a non-transgenic control, and MS3 corn treated with glufosinate ammonium. Although the firm described the analyzed forage as silage, it was green chop (the entire corn plant chopped at the dough stage of maturity prior to ensiling). The following analytes were measured: absolute dry matter; crude ash; nitrogen; crude fiber; crude protein; starch; phytic acid; amino acid composition; potassium, sodium, calcium, magnesium, phosphorus, phytate phosphorus; and *in vitro* organic matter digestibility. PGS concluded that the genetic modification did not change the composition of corn products intended for human or animal consumption.

Conclusions

PGS has concluded, in essence, that the corn line containing transformation event MS3 and its progeny are not materially different in composition, safety, and other relevant parameters from conventional corn varieties. At this time, based on PGS's description of its data and analyses, the agency considers PGS's consultation on corn grain (seeds) and silage, derived from corn lines containing transformation event MS3, to be complete.

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